# Binding and Diffusion of CheR Molecules Within a Cluster of Membrane Receptors

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ABSTRACT Adaptation of the attractant response in *Escherichia coli* is attributable to the methylation of its transmembrane chemotactic receptors by the methyltransferase CheR. This protein contains two binding domains, one for the sites of methylation themselves and the other for a flexible tether at the C terminus of the receptor. We have explored the theoretical consequences of this binding geometry for a CheR molecule associated with a cluster of chemotactic receptors. Calculations show that the CheR molecule will bind with high net affinity to the receptor lattice, having a high probability of being attached by one or both of its domains at any instant of time. Because of the relatively low affinity of its individual domains and the close proximity of neighboring receptors, it is likely that when one domain unbinds it will reattach to the array before the other domain unbinds. Stochastic simulations show that the enzyme will move through the receptor cluster in a hand-over-hand fashion, like a gibbon swinging through the branches of a tree. We explore the possible consequences of this motion, which we term "molecular brachiation", for chemotactic adaptation and suggest that a similar mechanism may be operative in other large assemblies of protein molecules.

## INTRODUCTION

The coliform bacterium *Escherichia coli* detects attractants and repellents in its environment by means of four homologous, dimeric receptors, Tar, Tsr, Trg, and Tap (Mowbray and Sandgren, 1998). These receptors are composed of an N-terminal periplasmic domain, a transmembrane region, and a long (~26 nm), coiled-coil cytoplasmic domain (Bass et al., 1999; Kim et al., 1999). The receptors tend to aggregate at the poles of the cell in a relatively stable complex with two other proteins of the chemotaxis signaling pathway, the histidine kinase, CheA, and the linking protein, CheW (Maddock and Shapiro, 1993). CheA autophosphorylates at a rate controlled by the ligand occupancy of the receptors, and acts as a phosphodonor for the response regulator, CheY. Phosphorylated CheY (CheYp) diffuses through the cytoplasm and binds to the switch complex at the base of the flagellar motor, thereby modifying the swimming behavior of the bacterium (Falke et al., 1997; Armitage, 1999; Bren and Eisenbach, 2000).

The receptors have four or five sites (glutamate or deamidated glutamine residues) in their cytoplasmic domains that undergo reversible methylation catalyzed by the methyltransferase, CheR, and the methylesterase, CheB (Mowbray and Sandgren, 1998; Zhulin, 2001). Together, these enzymes mediate adaptation of the chemotactic response by modifying the methylation state of the receptors to restore the activity of CheA (altered by the binding of ligands to the periplasmic domain of the receptors). In addition to interacting with the methylation sites midway along the cytoplasmic domain of the receptors, both CheR and CheB also interact with a second site at the extreme C terminus of the cytoplasmic domain (Wu et al., 1996). A crystallographic study has shown that the C-terminal pentapeptide of the major receptors, Tar and Tsr, binds to CheR in a subdomain remote from the active site (Djordjevic and Stock, 1998). Biochemical studies have shown that the presence of the pentapeptide is required for both CheR and CheB to work efficiently (Okumura et al., 1998; Barnakov et al., 1999; Shiomi et al., 2000). The pentapeptide is separated from C terminus of the cytoplasmic coiled-coil by a flexible tether (Le Moual and Koshland, 1996), which should enable either enzyme not only to modify sites on the receptor to which it is bound, but also on its immediate neighbors in a cluster of receptors. Direct experimental evidence for an interdimer mechanism of this kind has been obtained in the case of CheR, but not for CheB (Le Moual et al., 1997; Li et al., 1997).

In this study, we consider how the activity of CheR in particular (and by extension CheB) might be affected by its ability to bind to receptors at two distinct sites and by the tendency of chemotactic receptors to form large, lateral aggregates in the plasma membrane. The paper is divided into the following sections.

In the first section entitled Generic Model of a Brachiating Protein, we consider a generic model that illustrates a number of important features of the binding and diffusive movement of a bivalent "dumbbell" molecule, with two binding domains connected by a flexible linker, over a lattice of binding sites. We show that this dumbbell molecule will bind tightly to the lattice and yet undergo restricted diffusive motion within the cluster, moving by a novel hand-over-hand mechanism we term "molecular brachiation."

In the second section, Specific (CheR) Model, we provide a more specific model of the interaction of CheR molecules

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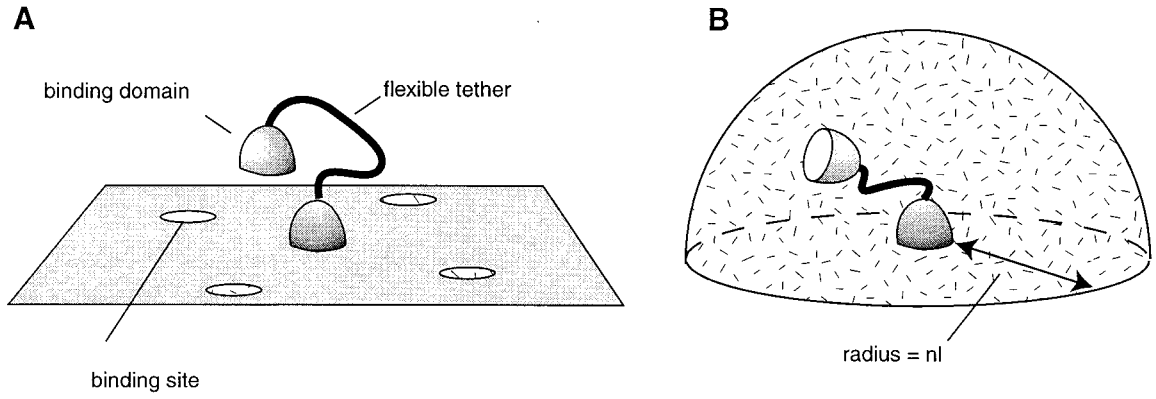


FIGURE 1 Cartoon of a generalized brachiating molecule. (*A*) The dumbbell-shaped molecule has two identical binding domains separated by a flexible tether and is shown bound by one of its domains to a single site on a planar lattice of sites. (*B*) Binding of the second domain is restricted to lattice sites that lie within the hemisphere swept out by the domain when the tether is fully extended (radius = $nl$, where $n$ is the number of amino acid residues in the tether and $l$ is the distance between successive $\alpha$-carbon atoms). The strength of association of this domain with the lattice will be controlled by the length and flexibility of the tether and by the spatial arrangement of the binding sites. However, it will be independent of the concentration of dumbbell molecules in free solution.

with the receptor lattice and show that, despite many differences, it nevertheless retains the essential features of a brachiating molecule, as seen in the case of the dumbbell protein. The many combinatorial possibilities arising in this situation are explored using a program that handles the interactions between individual molecules in a stochastic manner, from which we derive quantitative estimates of the movements of CheR molecules within the receptor lattice.

In the third section entitled Implications for Chemotaxis, we examine the implications of our analysis for the process of chemotactic adaptation and address the possibility of a variable binding affinity of CheR to the receptors and other complications in a nonquantitative manner. Finally, in section 4, Molecular Brachiation, we explore the more general implications of molecular brachiation for large arrays of protein molecules in other cells.

## GENERIC MODEL OF A BRACHIATING PROTEIN

Consider a hypothetical model in which a dumbbell-shaped molecule with two identical binding domains linked by a short, flexible tether, freely diffusing in aqueous solution, encounters a regular lattice of sites to which it can bind (Fig. 1). Initial contact will be through a conventional bimolecular binding and occur at a rate proportional to the concentration of freely diffusing molecules. However, once the first domain has attached to the lattice, then the effective concentration for binding of the second domain will depend only on the length and physical properties of the tether and on the positions of nearby lattice sites. Under suitable conditions, the "effective concentration" of this second domain may be very much higher than the concentration of freely diffusing molecules, and this will enhance the probability of the molecule existing in the doubly bound form.

How tight will the binding of the dumbbell molecule be? If we assume for simplicity that the two domains have identical (diffusion-limited) association rates, $k_{on}$, and dissociation rates, $k_{off}$, then, for the binding of unattached dumbbell molecules, concentration $R$, to a lattice of binding sites, concentration $T$, we have the following equilibrium conditions:

$$k_{on} \cdot 2R \cdot T = k_{off} \cdot \overline{TR}$$

$$k_{on} \cdot \overline{TR} \cdot \beta c_L = 2k_{off} \cdot \overline{TRT}$$

where $\overline{TR}$ and $\overline{TRT}$ are the concentrations of singly and doubly bound dumbbell molecules, respectively, and $c_L$ is the local concentration of each of $\beta$ neighboring binding sites (see Appendix). The effective dissociation constant for dumbbell molecules binding to the receptor lattice is therefore

$$\frac{R \cdot T}{\overline{TR} + \overline{TRT}} = \frac{1}{2(k_{on}/k_{off}) + (k_{on}/k_{off})^2 \beta c_L} \cong \frac{K_d^2}{\beta c_L} \quad (1)$$

where $K_d = k_{off}/k_{on}$ is the dissociation constant of each domain of the dumbbell molecule.

To estimate the effective binding coefficient of the dumbbell molecule to the lattice, we therefore need estimates for $K_d$, $\beta$, and $c_L$. Taking values similar to those calculated for the CheR situation (see Specific (CheR) model), we used $K_d = 2\ \mu$M, $\beta = 6$, and $c_L = 0.17$ mM to obtain an apparent dissociation constant very close to 4 nM. In Fig. 2, we show the occupancy expected for a range of concentrations of dumbbell molecule and for the same molecule in which the flexible tether has been cut. Under certain conditions, in particular if the dumbbell molecules are present in excess, the bivalent binding leads to a dramatically enhanced occupancy of the receptor lattice.

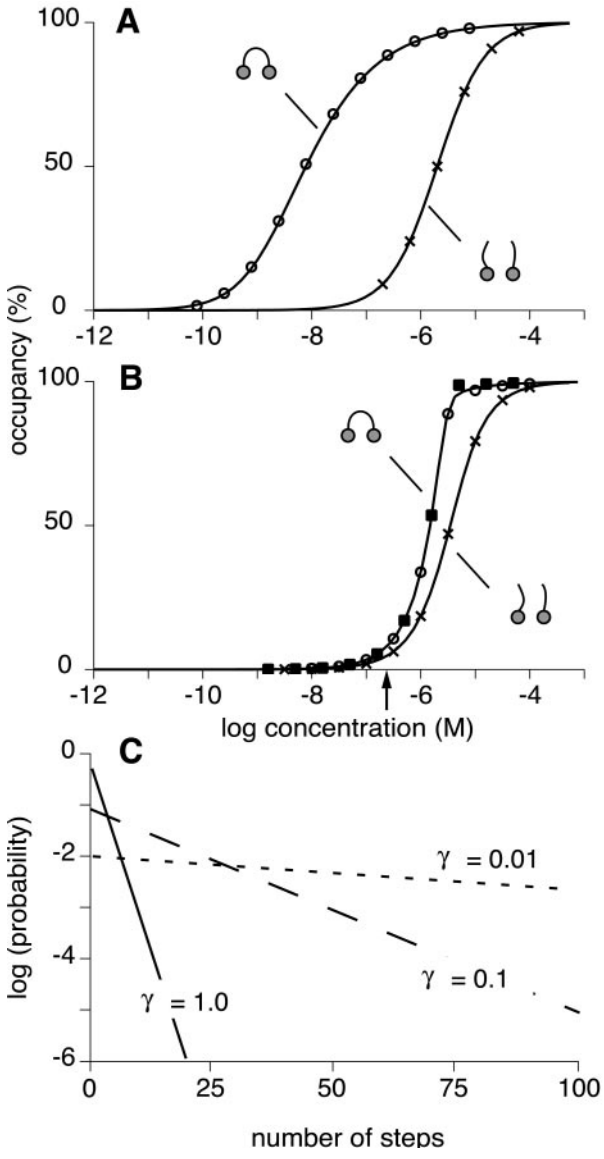


FIGURE 2 Binding and diffusion of a dumbbell molecule. In this case, the tether is composed of 30 amino acids, the characteristic ratio (Appendix), $C_n = 5$, and the distance between binding sites on the lattice, $r = 5\sqrt{2}$ nm. The $K_d$ for the binding of individual binding domains is 2 $\mu$M and the concentration of binding sites in the lattice, [T] = 2.94 $\mu$M. (*A*) Effect of the flexible tether on the binding of a dumbbell molecule, if the latter is present in excess (i.e., the lattice is exposed to an infinitely large volume of solution containing dumbbell molecules). Changes in occupancy with ligand concentration, given by $[TR] = [R]/([R] + K_d)$, where [R] and [*TR*] are the concentrations of free and bound dumbbell molecules, respectively, are shown for an intact dumbbell molecule and for the same molecule in which the linker has been cut. The intersection with the horizontal dotted line indicates the concentration necessary to give 50% occupancy of the lattice. The continuous curves were obtained by numerical integration of the binding equations given in the text, with the number of nearest neighbors at each site ($\beta$), initially six, falling linearly to zero as the occupancy of the lattice by the dumbbell molecule rises. Discrete points (○ and ×) show the results of stochastic simulations with the same parameters (see text for details). (*B*) As for (*A*) but with the reactions occurring in a finite volume. In this case, the change in occupancy with ligand concentration is given by $[TR] = 0.5\{[T] + [R] + K_d - \sqrt{([T] + [R] + K_d)^2 - 4[T][R]}\}$. The results of stochastic simulations of the CheR-receptor system (see text for details) are also shown (■) with the arrow indicating the physiological concentration of CheR. (*C*) The probability of the dumbbell molecule completing a series of *s* steps across the lattice before detaching. As discussed in the text, the distance traveled depends on the ratio, $\gamma$, of the dissociation constant of the individual binding domains, $K_d$, to the effective local concentration of the tethered domain, $c_L$, at $\beta$ neighboring binding sites (Eq. 2).

We could, of course, achieve a similar degree of selective binding if the diffusing molecule had a single, high-affinity binding domain with a $K_d$ of 4 nM. However, this would have the necessary corollary that the molecule would remain immobilized at individual lattice sites for extended periods of time. With a diffusion-limited rate of protein-protein association of $5 \times 10^6$ $M^{-1}$ $s^{-1}$ (Northrup and Erickson, 1992; Camacho et al., 2000), the average dwell time of a molecule bound with a $K_d$ of 4 nM is ~50 s. Each domain of the dumbbell molecule, by contrast, will dissociate at a rate commensurate with a $K_d$ of 2 $\mu$M and have an average dwell time of 0.1 s. Evidently, a dumbbell molecule with the same effective $K_d$ of 4 nM will be more mobile and able to move around on the lattice much more rapidly.

How far will it move before detaching? We can examine this question by a simple consideration of probabilities. If we start with a molecule that is doubly attached, then the next event must be the detachment of one domain, that is, the probability of this event $P(2\rightarrow1) = 1$. We then have two possibilities: either the same domain will reattach (1→2), in which case the molecule will have taken a single "step", or the remaining domain will detach (1→0), which will cause the molecule to diffuse away and terminate the sequence of steps. Because we must have one of these two events:

$$P(1\rightarrow2) + P(1\rightarrow0) = 1$$

The ratio of these probabilities will be given by the ratio of the two rates:

$$P(1\rightarrow2)/P(1\rightarrow0) = k_{on}\beta c_L/k_{off} = \beta c_L/K_d$$

Thus, the probability of each step,

$$p = P(2\rightarrow1)P(1\rightarrow2) = \beta c_L/(\beta c_L + K_d)$$

and the probability of a chain of *s* steps is $p^s(1 - p)$ or, in exponential form,

$$(1 - p)\exp[s \cdot \ln(p)] = \frac{\gamma}{1 + \gamma}\exp[-s \cdot \ln(1 + \gamma)] \quad (2)$$

where $\gamma = K_d/\beta c_L$.

Because the direction of each step is random, the dumbbell molecule will move in a diffusive fashion over the receptor lattice with an effective diffusion coefficient of

$$\frac{\langle(\Delta x)^2 + (\Delta y)^2\rangle}{4\Delta t} = \frac{\langle r_d^2}{4\Delta t} \quad (3)$$

Plots of the distance traveled by a brachiating molecule for a range of values of $\gamma$ are shown in Fig. 2 *C*.

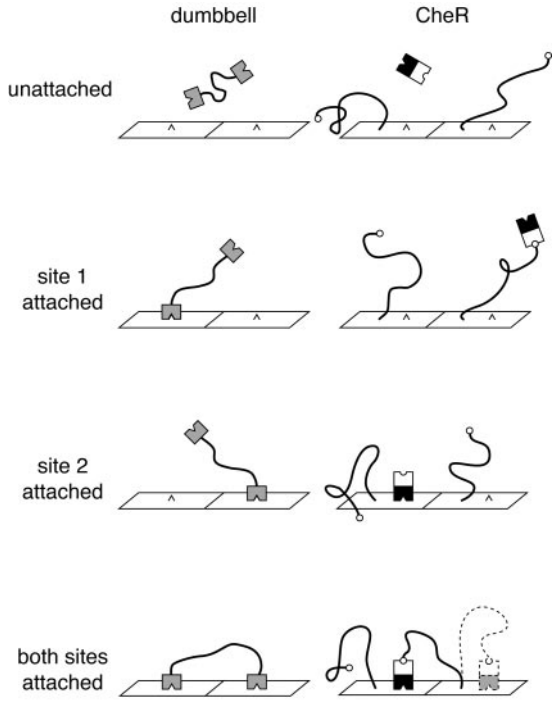


FIGURE 3 CheR as a brachiating molecule. Comparison of generic dumbbell molecules and CheR molecules in a lattice of chemotactic receptors emphasizes the formal similarity between the two. Note that the presence of two binding sites on the major chemotactic receptors allows both inter- and intrareceptor binding of CheR.

## SPECIFIC (CheR) MODEL

We now turn to the methylating enzyme CheR and its association with the cluster of chemotactic receptors. Clearly, this differs in a number of respects from the case just considered. CheR molecules are not dumbbells and their two domains are not equivalent. Moreover, the receptors have binding sites of two kinds that lie in parallel planes: 1) a C-terminal pentapeptide and 2) a region containing the methylation sites. Nevertheless, there is a formal analogy between the two situations, because in both there are two binding interactions per molecule, and one is enhanced by the limited diffusion of a flexible stretch of polypeptide chain (Fig. 3). Under suitable conditions, therefore, CheR could behave like the hypothetical dumbbell molecule and brachiate within the receptor lattice. To evaluate this possibility, we need to examine in more detail the lattice of receptors and their interactions with CheR molecules.

Estimates of the total number of transmembrane chemotactic receptors present in coliform bacteria range from 1500 to 4000 dimers per cell (DeFranco and Koshland, 1981; Hazelbauer and Harayama, 1983; Gegner et al., 1992). Most receptors form stable ternary complexes with CheA and CheW that aggregate into higher-order structures, predominantly at one of the poles of the cell (McNally and

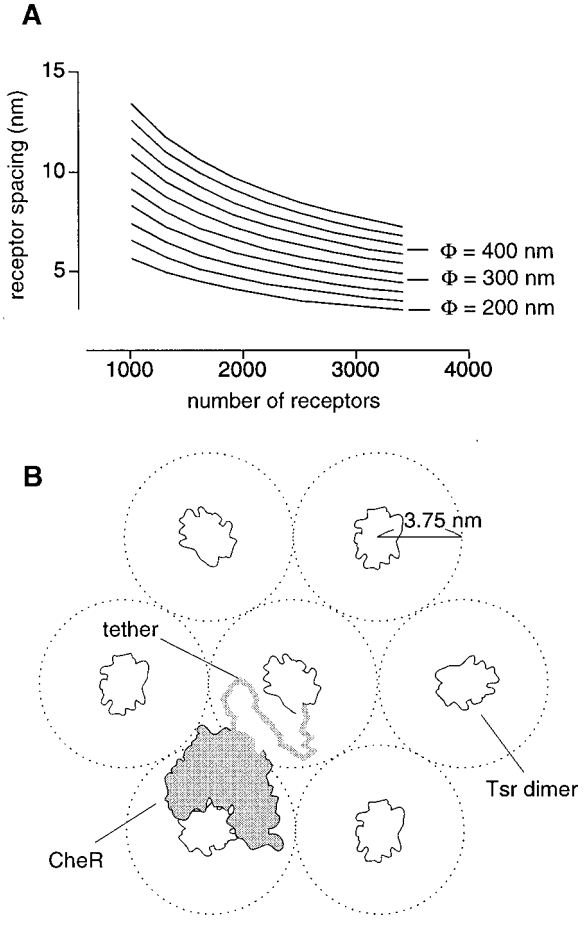


FIGURE 4 Schematic of the receptor cluster. (*A*) Estimates of the average distance between receptors in a circular cluster with total diameter $\phi$ nm containing $n$ receptor dimers. Calculated from $d = \alpha\phi/\sqrt{n}$ with $\alpha = 0.9$. Note: $\alpha = 0.89$ for square packing, and $\alpha = 0.95$ for hexagonal packing. Calculations in this paper are based on a receptor-receptor distance of 7.5 nm. (*B*) Localization of CheR in a hexagonally packed array of receptors. Cross-sectional contours of the receptor dimer and CheR were visualized using PDB files with accession codes 1QU7 (Kim et al., 1999) and 1BC5 (Djordjevic and Stock, 1998), respectively. The center-to-center spacing of the receptor dimers is 7.5 nm.

Matsumura, 1991; Gegner et al., 1992; Maddock and Shapiro, 1993; Schuster et al., 1993; Liu et al., 1997; Gestwicki et al., 2000). The clusters are typically up to 500 nm in diameter (Maddock and Shapiro, 1993; Gestwicki et al., 2000), leading to a range of possible receptor-receptor spacings (Fig. 4 *A*). In this study, we have taken a value of 7.5 nm, which gives a receptor density similar to that predicted in an atomic-level model of the Tsr receptor lattice proposed by Shimizu et al. (2000).

The methyltransferase CheR transfers a methyl group from *S*-adenosylmethionine to specific glutamate (and deamidated glutamine) residues on the chemotactic receptors Tar, Tsr, Tap, and Trg (Djordjevic and Stock, 1997). The kinetic mechanism of the enzyme is random with respect to the binding of the substrates to form the ternary complex

(Simms and Subbaramaiah, 1991). The number of CheR molecules per *E. coli* cell has been estimated to be ~200, that is approximately one-tenth the number of receptors (Simms et al., 1987). Analysis of the crystal structure of CheR (PDB accession code 1AF7 (Djordjevic and Stock, 1997)) reveals it to be an L-shaped protein composed of two globular domains, with overall dimensions $7 \times 6 \times 4$ nm$^3$. The C-terminal domain carries both the catalytic site and a subdomain that binds the C-terminal pentapeptides of Tar and Tsr (PDB accession code 1BC5 (Djordjevic and Stock, 1998)) with a dissociation constant of 2 $\mu$M (Wu et al., 1996). There are no published estimates for the binding strength of the catalytic site to the receptor and we have assumed for simplicity that this $K_d$ is also 2 $\mu$M. The possibilities that this $K_d$ is higher than 2 $\mu$M (that is, weaker binding), or that it varies with the methylation or conformational state of the receptor, are discussed in the section Implications for Chemotaxis.

The flexible tethers extending from the central coiled-coil of chemotactic receptors vary in both length and sequence between different receptor types and different species. In the case of the *E. coli* serine receptor, the tethers are ~30 residues long (excluding the five C-terminal residues involved in binding CheR) (Le Moual and Koshland, 1996). A polypeptide chain of this size has a maximum extended length of 11.4 nm, but the root-mean-square end-to-end distance at any instant of time will be much less than this, because of the dynamic twisting and flexing of the chain within the confines of the receptor lattice (Appendix). If the receptor lattice is a hexagonally packed array with a center-to-center receptor spacing of 7.5 nm, then each receptor will have six nearest neighbors with their surfaces lying 5 nm apart (taking the receptor dimers to be cylinders of diameter 2.5 nm) (Fig. 4 *B*). For simplicity, we assume that there is a single tether and methylation site per receptor dimer, with the methylation site 5 nm along the coiled-coil (toward the interior of the cell) from the start of the tether. A distance of 5 nm is therefore spanned when CheR is bound to both the tether and the methylation site of the same receptor, but $5\sqrt{2}$ nm when bound to the tether of one receptor and the methylation site of one of its neighbors.

The previously described stochastic simulation program StochSim (Morton-Firth and Bray, 1998) was used to model the interactions between CheR molecules in the cytosol and the receptor lattice. The version of the program used (available from ftp://ftp.cds.caltech.edu/pub/dbray) includes a two-dimensional (2-D) spatial representation, enabling interactions between neighboring receptors in the lattice to be followed (Le Novère and Shimizu, 2001). In this formulation, molecules not associated with the lattice are assumed to be in a uniformly mixed solution. CheR molecules were allowed to bind to a hexagonally packed array of 2500 receptor dimers (~3 $\mu$M), and the rate of the binding reaction was modified according to whether one domain of the brachiating molecule was already bound to the array or not, taking into account the effect of the flexible tether on local concentration for both intra- and interreceptor modes of binding.

The $K_d$ of each binding site on the receptor (C-terminal pentapeptide and methylation site) was assumed to be 2 $\mu$M, and the effective local concentrations attributable to restricted diffusion of the tether were 0.96 mM and 0.17 mM for intra- and interreceptor binding, respectively, with a characteristic ratio of 5.0 (Appendix). Stochastic simulations with these parameters show that the effective $K_d$ for binding of CheR to the receptor array is ~1.6 nM (using Eq. 1, analogous dumbbell models would give values of 0.7 and 3.9 nM, respectively). At physiological concentrations, almost all (99.9%) of the CheR molecules in the cell would be associated with the receptors cluster with this strength of binding.

Stochastic simulations were used to examine the diffusion of CheR molecules within the receptor array. These showed that molecular brachiation provides an efficient way for a CheR molecule to visit the available sites in a local area. The path traced out by the molecule tends to cover dense patches of sites on the 2-D array in contrast to the pepper-and-salt pattern generated by a single-binding-domain molecule (Fig. 5). Both simulations are somewhat unrealistic, however, in that they do not take into account the fact that when a reversibly bound ligand dissociates from a receptor, it tends to rebind to the same or a nearby receptor after diffusing for a time in the immediate vicinity (Berg and Purcell, 1977; Lagerholm and Thompson, 1998). This effect should increase the tendency of the sites where the single-binding-domain molecule in Fig. 5 rebinds to form small clusters, but it also applies to the brachiating molecule during its detachment and rebinding between brachiation paths. Other simplifications used in constructing the model of CheR brachiation are discussed in the section Implications for Chemotaxis. When a CheR molecule in the simulation encountered a boundary of the receptor cluster, it was, in effect, reflected from that boundary (Fig. 5 *A*). This behavior, which is a direct consequence of the brachiation mechanism, ensures that the CheR molecule will remain associated with the receptor cluster for extended periods of time.

## IMPLICATIONS FOR CHEMOTAXIS

There is no direct evidence for the movement of CheR by brachiation through clusters of chemotactic receptors. The most unequivocal proof would require the visualization of individual CheR molecules in a cluster and observation of their residency and diffusive motion. This would certainly be difficult, not only because of the minute size of the clusters but also their inaccessibility. One could hope to produce larger clusters in cells, perhaps on those generated by inhibitors of septation (Maki et al., 2000), or even to reconstruct large clusters in vitro by the self-assembly of

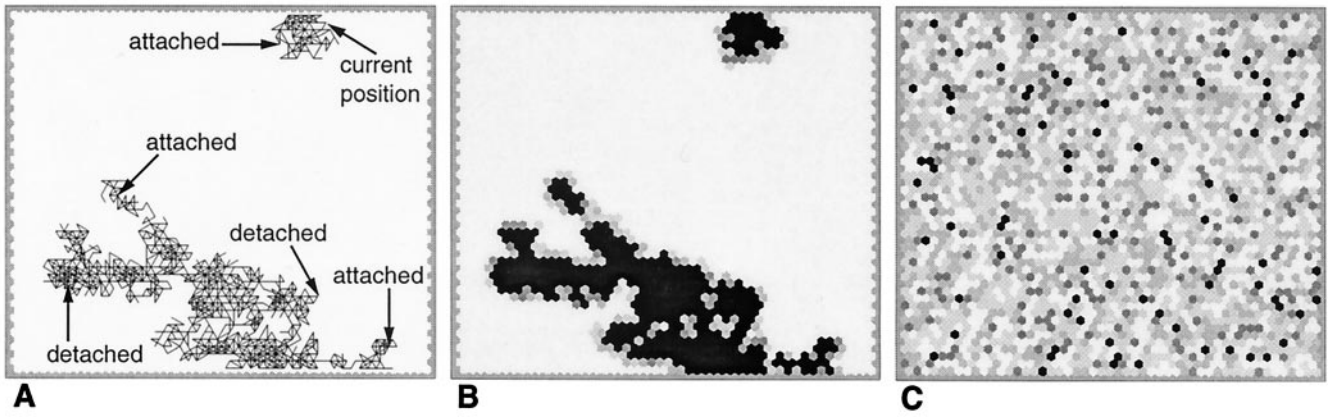


FIGURE 5 Stochastic simulation of brachiation. A single CheR molecule in a volume of 1.4 fl (the approximate volume of a bacterial cell) was allowed to diffuse to a lattice of binding sites and followed over a period of 500 s. (*A*) Loci of the CheR molecule on the surface of the lattice during the simulation. The molecule moves across the lattice by brachiation with an effective diffusion coefficient of $1.66 \pm 0.04 \times 10^{-13}$ cm$^2$ s$^{-1}$ (Eq. 3), its position being sampled at 0.1-s intervals. During this run, the molecule detached twice from the surface after the initial binding and then re-attached, resulting in the three brachiation paths shown. Note that the molecule tends to "rebound" when it encounters the lattice boundary, in effect being trapped by the array. (*B*) Coverage of the lattice by the CheR molecule. Binding sites visited by the molecule are shown in shades of gray, with the intensity indicating the number of repeat visits (1, 2, 3, $\geq$4). (*C*) As for (*B*) but with the tethers on the receptors removed, so that each receptor has only one binding site for CheR. Brachiation does not occur under these conditions.

proteins in an artificial membrane. Membrane fractions from bacteria are indeed routinely used in the analysis of chemotaxis function, but the chemotactic receptors comprise a small fraction of the total membrane protein and the extent of clustering in the absence of cytoplasmic components remains uncertain.

There is, however, indirect or circumstantial evidence that all the conditions are in place in the living cell for brachiation of CheR to occur. To recapitulate, CheR has two distinct binding sites for the receptor: one that binds the C terminus and one that recognizes the glutamate residues that undergo methylation. Moreover, the C-terminal pentapeptide involved in binding is situated at the end of a sequence of amino acids (estimated to be ~30 residues long) that appears to have no regular structure. In such a situation, provided that the arrangement of receptor dimers in the cluster is reasonably regular and the separation between adjacent receptors is not too great, a CheR molecule attached to this site should be capable of reaching a neighboring receptor in the cluster and binding to it with enhanced affinity. Consistent with this view, there is direct biochemical evidence that CheR can catalyze the modification of one receptor while being anchored to a neighboring receptor through the flexible tether (Le Moual et al., 1997; Li et al., 1997). Moreover, it has been shown that deletion of part or all of the tether, which should abolish brachiation, reduces the activity of CheR by between one and two orders of magnitude (Le Moual et al., 1997; Li et al., 1997; Barnakov et al., 1999). If detachment and re-attachment of a CheR molecule occur at its two sites independently but at similar rates, there will be an opportunity, during episodes in which it is attached at just one site, for diffusive movement to occur. In other words, the CheR molecule could move from receptor to receptor in a manner reminiscent of a gibbon swinging through the branches of a tree, hence the term molecular brachiation.

What will be the consequences of this novel motion for chemotaxis? One salient feature of the proposed diffusive motion is that the time spent by a CheR molecule in contact with an individual receptor will be relatively brief. If the two binding sites have identical values of $K_d$ of 2 $\mu$M, then the time spent in association with either site would average 0.1 s. Interestingly, this duration matches quite well the time taken to add a methyl group to a receptor, as purified CheR in vitro has a $V_{max}$ of ~10 methyl groups per receptor per minute (Simms et al., 1987) (and estimates based on the in vivo rate of adaptation give a similar value). In broad terms, therefore, the mechanism of brachiation should allow a CheR molecule time to methylate each receptor to which it binds before moving on to a different one. A simple binding interaction through a high-affinity site, by contrast, would be expected to produce a much longer dwell time. With a single binding site with a $K_d$ of 4 nM, an enzyme would remain attached to an individual receptor for 50 s or so, which is incompatible with the observed catalytic rate.

Much of what has been said so far regarding CheR could also be applied to the demethylating enzyme CheB. This similarly sized enzyme also has two binding sites, one for a methylated residue on a receptor and the other for the C-terminal flexible tether. Although details of the interaction differ (Barnakov et al., 2001), it seems likely that CheB could also work in both an intra- and an interdimer manner, allowing it to brachiate through the receptor cluster. Another difference relates to the number of CheB molecules in

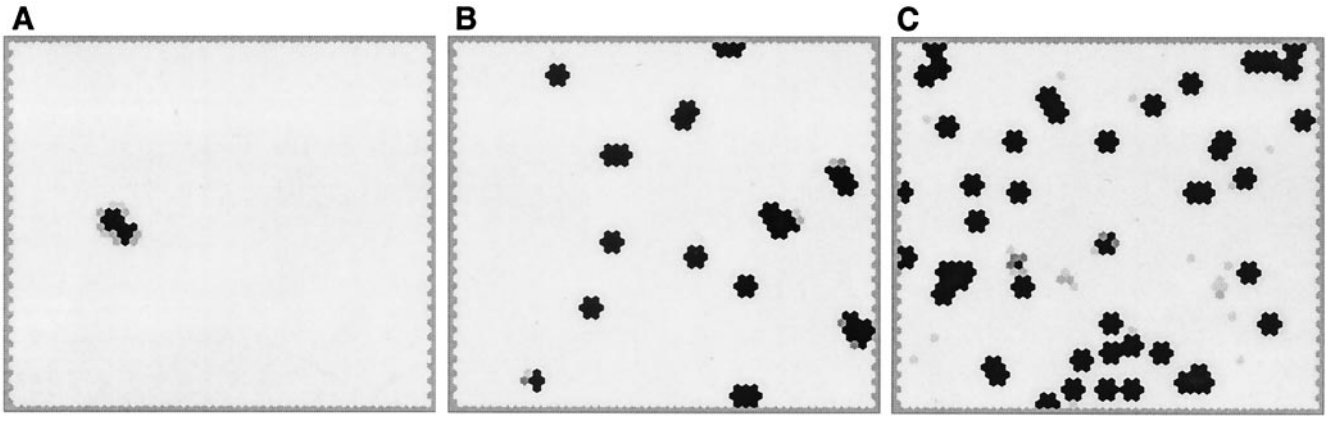


FIGURE 6 Brachiation with asymmetric values of $K_d$. Coverage of the lattice by the CheR molecule over 10 s with a $K_d$ for the C-terminal flexible tether of 2 $\mu$M and for the methylation site of (*A*) 2 $\mu$M (equal binding affinities), (*B*) 0.2 mM (100-fold weaker binding), and (*C*) 2 mM (1000-fold weaker binding). See legend of Fig. 5 for details.

the cell, apparently some 10 times higher than that of CheR (Simms et al., 1985). However, given that CheB is activated by autophosphorylation (using phosphorylated CheA as a phosphodonor), it may be that the number of active CheB molecules in a cell is not very different from the number of CheR.

There is no doubt that the actual situation in a real cluster of receptors is much more complicated than that portrayed here. Not only are there two flexible tethers and eight or 10 methylation sites per receptor dimer, but the clusters themselves are likely to be irregular in size and shape and to include receptors of different types, Tar, Tsr, Trg, and Tap, mixed together. Furthermore, as the low-abundance receptors Trg and Tap lack the C-terminal flexible tethers present on Tar and Tsr, they would be unable to mediate movement by brachiation. In fact, it has been suggested that the efficient methylation and demethylation of the low-abundance receptors will rely on their close proximity to the more abundant Tar and Tsr (Feng et al., 1997; Weerasuriya et al., 1998; Feng et al., 1999). Evidently, the presence of low-abundance receptors in the lattice will modify, and perhaps disrupt, the smooth progression of a brachiating molecule across the lattice.

An even more fundamental complication is introduced by the conformational changes undergone by receptors in response to ligand binding, which are thought to be the basis of signal transduction in this system (Falke and Hazelbauer, 2001). It is widely believed that CheR methylates only receptors in the conformation that *inactivate* CheA (a situation favored by attractant binding) (Terwilliger et al., 1986; Shapiro et al., 1995), whereas CheB demethylates only receptors in the conformation that *activate* CheA (a situation favored by repellent binding) (Borczuk et al., 1986; Sanders and Koshland, 1988). If this scenario is correct, then 1 of the 2 binding domains of CheR (the one associated with the catalytic site) is likely to show a variable $K_d$ for receptors depending upon their current conformational state and (perhaps) degree of methylation. If, moreover, the binding of CheR or CheB to a receptor actually stabilizes a particular conformation, then these two enzymes will tend to exclude one another and perhaps form local domains of different methylation state within the field of receptors.

In this study, we have assumed for simplicity that the affinity of CheR for the methylation site is the same as for the flexible tether, although physiologically they are likely to differ. If the affinity for the methylation site is weaker, for example, then the higher the value of this $K_d$, the lower the overall affinity of a CheR molecule for the receptor lattice will be and the more limited in extent its brachiation. Under such conditions, the molecule would be expected to remain attached to an individual tether as it diffuses between the methylation sites in its vicinity. Movement to a new tether would be possible but infrequent, because of the lower affinity of the second site. In a preliminary investigation of this situation, we performed simulations in which values of $K_d$ of the methylation binding site were 100- and 1000-fold higher (that is, 0.2 and 2 mM, respectively), large enough for any differences in behavior to become apparent (Fig. 6). As expected, a CheR molecule in these circumstances becomes more restricted in its ability to diffuse over extended regions within the cluster.

## MOLECULAR BRACHIATION

Although we are not aware that the concept of molecular brachiation has been described previously, phenomena of a similar kind have been extensively documented. The existence of "interdomain linkers", or flexible tethers, in bacterial two-component regulatory systems was noted over a decade ago (Wootton and Drummond, 1989), and a number of examples are known in which their use results in enhancements in local

concentration. A particularly well characterized system is the *Shaker* $K^+$ channel, which has been subject to both experimental and theoretical investigation (Hoshi et al., 1990; Timpe and Peller, 1995). Inactivation of the channel occurs when an N-terminal regulatory domain, attached to the core of the protein by a linker assumed to be free of regular secondary-structure elements, physically blocks the opening of the pore. Flexible looping is also a feature of protein-DNA interactions, where it serves to bring proteins attached at distant sites, such as those involved in transcriptional regulation, close together (Dröge and Müller-Hill, 2001). In eukaryotes, the movement of bivalent molecules along cytoskeletal filaments enables organelles and vesicles to be transported from one location in the cell to another. The agents in this case are two-headed motor proteins such as kinesin or myosin IV, which couple the hydrolysis of ATP to unidirectional motion along the filament. However, it is likely that such molecules would, in the absence of ATP hydrolysis, undergo diffusive random walks or "1-D brachiation" (Vilfan et al., 2001). Moving to three dimensions, we might mention the fact that networks of actin filaments in the membrane cortex of many eukaryotic cells typically contain a variety of bivalent actin-binding proteins. Some of these, such as $\alpha$-actinin and filamin, have two identical actin-binding domains linked by a flexible tether and so should be able to brachiate within the actin meshwork.

In summary, we suggest that the mechanism of molecular brachiation introduced here could operate in a variety of situations within the cell. It carries the potential advantage of allowing an enzyme or other active molecule to be sequestered to a large structure, without the concomitant requirement that the molecule also becomes effectively immobilized because of high-affinity binding. As shown in this study, a brachiating molecule can move in a diffusive fashion over the surface of a 2-D lattice and thereby spread its activity over the entire structure in a relatively short period of time. This physically realistic property should provide cells with the ability to control self-assembly processes in a sensitive and highly flexible manner.

## APPENDIX

The random configurations that chain molecules adopt in solution mimic 3-D random walks (or flights) with steps of fixed length. For long chains, the local concentration (in molecules per unit volume) of the free end at a location a distance $r$ from the fixed end follows the Gaussian distribution given by

$$\left(\frac{3}{2\pi\langle r^2\rangle}\right)^{3/2}\exp\left(-\frac{3r^2}{2\langle r^2\rangle}\right) \tag{A1}$$

where $\langle r^2\rangle$ is the mean square end-to-end distance (Flory, 1969; Timpe and Peller, 1995). For a freely jointed chain with $n$ bonds of fixed length $l$ and no constraints on the orientation of successive bonds,

$$\langle r^2\rangle = nl^2 \tag{A2}$$

For polypeptide chains, however, there is a degree of correlation between the orientation of successive residues because of the geometry of carbon atoms and steric hindrance from the side chains of the residues. This is usually expressed as the characteristic ratio $C_n$, which quantifies the deviation from the ideal chain represented by Eq. A2:

$$C_n = \langle r^2\rangle/nl^2 \tag{A3}$$

$C_n$ is an increasing function of $n$, but for very long chains approaches the limit, $C_\infty$ (considered to be a measure of the "stiffness" of the chain), and lies in the range 8.5–9.5 for several synthetic homopolypeptides (Brant and Flory, 1965). Substituting Eq. A3 into Eq. A1, the expression for the local concentration of the free end of a tether becomes

$$\left(\frac{3}{2\pi C_n nl^2}\right)^{3/2}\exp\left(-\frac{3r^2}{2C_n nl^2}\right) \tag{A4}$$

in terms of molecules per cubic nm, or

$$c_L = \left(\frac{3}{2\pi C_n nl^2}\right)^{3/2}\exp\left(-\frac{3r^2}{2C_n nl^2}\right)\Big/10^{-24}N_A \tag{A5}$$

in moles per liter, where $N_A$ is Avogadro's number. Taking the distance between successive $\alpha$ carbon atoms, $l$, as 0.38 nm, this expression then simplifies to

$$c_L = 9.98(C_n n)^{-3/2}\exp(-10.4r^2/C_n n) \tag{A6}$$

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